

1

# MODIFIED RELAXIN POLYPEPTIDES COMPRISING A PHARMACOKINETIC ENHANCER AND USES THEREOF

## RELATED APPLICATION DISCLOSURES

This application claims the benefit of U.S. Provisional Application 62/627,411, filed Feb. 7, 2018 and U.S. Provisional Application No. 62/456,161, filed Feb. 8, 2017, the disclosure of all of which listed above are herein incorporated by reference in their entirety.

## SEQUENCE DISCLOSURE

This application includes as part of its disclosure a biological sequence listing, contained in a file named "46561o2401.txt" having a size of 150,746 bytes, which was created on Feb. 8, 2018, which is hereby incorporated by reference in its entirety.

## BACKGROUND OF THE DISCLOSURE

The present disclosure generally relates to modified relaxin polypeptides, such as modified human relaxin 2 polypeptides, comprising a pharmacokinetic enhancer, and therapeutic uses of such polypeptides, such as for the treatment of cardiovascular conditions (such as heart failure) and/or conditions relating to fibrosis. In exemplary embodiments, the pharmacokinetic enhancer is linked to a non-naturally encoded amino acid, which may be ribosomally incorporated into the relaxin polypeptide.

Heart failure (HF) represents a tremendous burden on today's health care system with an estimated United States prevalence of 5.8 million and greater than 23 million worldwide (Roger et al., 2012. *Circulation*, 125(1): e2-e220). The symptoms of HF are the result of inadequate cardiac output and can be debilitating depending upon the advanced stage of the disease. Major symptoms and signs of HF include: 1) dyspnea (difficulty in breathing) resulting from pulmonary edema due to ineffective forward flow from the left ventricle and increased pressure in the pulmonary capillary bed; 2) lower extremity edema occurs when the right ventricle is unable to accommodate systemic venous return; and 3) fatigue due to the failing heart's inability to sustain sufficient cardiac output to meet the body's metabolic needs (Kemp & Conte, 2012. *Cardiovascular Pathology*, 21:365-371).

Many contributory diseases, risk factors, and pathological changes may ultimately lead to heart failure (Jessup & Brozena, 2003. *N Engl J Med*, 348(20): 2007-2018). Injurious events thought to be involved in the pathophysiology of HF range from the very acute such as myocardial infarction to a more chronic insult such as life-long hypertension. The death rate remains high with ~50% of people with HF dying within 5 years of diagnosis (Roger et al., 2012. *Circulation*, 125(1): e2-e220; Roger et al., 2004. *Jama*, 292(3): 344-50). Heart failure clearly presents a significant unmet medical need.

The human relaxin 2 hormone (also called H2 relaxin) is a 6-kDa peptide composed of 53 amino acids which was known to be responsible for remodeling the reproductive tract before parturition, thus facilitating the birth process. While predominantly a hormone of pregnancy, relaxin has also been detected in the non-pregnant female as well as in the male. Human relaxin is a member of the insulin peptide family which includes insulin, a number of insulin like peptides (INSL3-6), and the insulin-like growth factors (IGFI and IGFI) (Van Der Westhuizen et al., 2007. *Curr*

2

*Drug Targets*, 8(1): 91-104). These heterodimeric peptides are all structurally related with each comprised of two peptide chains (A & B) that are connected by two disulfide bonds, and with the A-chain containing a single intramolecular disulfide bond. The receptor for relaxin 2 (H2), called the Relaxin Family Peptide Receptor 1 (RXFP1), is conserved between mouse and human with 85% amino acid identity and is essentially ubiquitously expressed in humans and in other species (Halls et al., 2007. *Br J Pharmacol*, 150(6): 677-91).

During human gestation, in order to meet the nutritional demands imposed upon it by the fetus, the female body undergoes a significant ~30% decrease in systemic vascular resistance (SVR) and a concomitant ~50% increase in cardiac output (Jeyabalan, 2010: *Renal and Electrolyte Disorders*. Lippincott Williams & Wilkins. 462-518; Clapp and Capeless, 1997. *Am J Cardiol*, 80(11): 1469-73). Additional vascular adaptations include an ~30% increase in global arterial compliance that is important for maintaining efficient ventricular-arterial coupling, as well as an ~50% increase in both renal blood flow (RBF) and glomerular filtration rate (GFR), important for metabolic waste elimination (Jeyabalan, 2010: *Renal and Electrolyte Disorders*. Lippincott Williams & Wilkins. 462-518; Poppas et al., 1997. *Circulation*, 95(10): p. 2407-15). Both pre-clinical studies in rodents as well as clinical studies performed in a variety of patient settings provide evidence that relaxin is involved, at least to some extent, in mediating these adaptive physiological changes (Conrad, 2011. *Am J Physiol Regul Integr Comp Physiol*, 301(2), R267-275; Teichman et al., 2009. *Heart Fail Rev*, 14(4), 321-329). Many of these adaptive responses would likely be of benefit to HF patients in that excessive fibrosis, poor arterial compliance, and poor renal function are all characteristics common to heart failure patients (Mohammed et al., 2015. *Circulation*, 131(6), 550-559), (Wohlfahrt et al., 2015. *Eur J Heart Fail*, 17(1), 27-34; Dammon et al., 2011. *Prog Cardiovasc Dis*, 54(2), 144-153). As an estimated 30% of patients with HF suffer from moderate to severe renal impairment (Tripodskiadis and Skoularigis, 2012. *Curr Heart Fail Rep*, (4):354-62), an agent such as relaxin by improving both vascular flow and electrolyte handling, may be of particular benefit to HF patients.

The relaxin peptide has a short pharmacokinetic half-life: Serelaxin, a recombinant human relaxin peptide, which was developed for the treatment of HF, has a short first-phase pharmacokinetic half-life of 5-15 minutes, and necessitated 48 hours continuous intravenous infusion for therapeutic utility (REASANZ (serelaxin) Briefing Document Prepared by Novartis for FDA Cardiovascular and Renal Drugs Advisory Committee Meeting. Feb. 26, 2014). For chronic diseases like heart failure, a relaxin molecule with an improved pharmacokinetic profile provides the opportunity for alternate routes of drug administration, beyond continuous intravenous infusion, likely to be more amenable as a therapeutic for patients suffering from chronic diseases.

## SUMMARY OF THE DISCLOSURE

Provided herein are modified relaxin polypeptides comprising a non-naturally encoded amino acid, wherein (a) the relaxin polypeptide comprises the relaxin A chain polypeptide of SEQ ID NO: 4 and the relaxin B chain polypeptide of SEQ ID NO: 5 or SEQ ID NO: 6, substituted with a non-naturally encoded amino acid at a position selected from the group consisting of: A chain residue 1, A chain residue 2, A chain residue 5, A chain residue 13, A chain